VASCULAR EFFECTS OF THE BUFODIENOLIDES

JULES B. PUSCHETT, M.D., FACP, FASN, FAHA, FAAAS

TEMPLE, TX

ABSTRACT

The bufodienolides are natriuretic steroids, which also have the capacity to cause vasoconstriction, and are cardiac inotropes. Their mechanism of action appears to be related to their ability to inhibit Na^+/K^+ ATPase. The actions of one of these compounds, marinobufagenin (MBG), have been investigated in a rat model of preeclampsia, an example of volume expansion-mediated hypertension. The urinary excretion of MGB is increased in this model. Furthermore, this increment in its excretion occurs *prior* to the development of hypertension and proteinuria. The animals also demonstrate intrauterine growth restriction. Studies of the effect of MBG on cytotrophoblast cells reveal that MGB inhibits the migration, proliferation and invasion of these cells. We propose that MGB is an important etiologic factor in at least some forms of preeclampsia and that the level of its excretion in the urine may prove to be of diagnostic value.

My colleagues and I have been fascinated for some time with the phenomenon of volume expansion. If, for example, one infuses a liter of saline, how does the body know how to handle it? It turns out that in the normal subject or in the animal, the resultant natriuresis represents a complex set of interactions involving physical factors and neurohumoral actions. More recently, our preoccupation with volume expansion has led us to an evaluation of volume expansion-mediated hypertension. Let me digress for a moment to remind you about some important background concepts.

Shown in Figure 1 is the relationship between blood flow (Q), blood pressure (P) and resistance to flow (R) in the vascular circuit. If we solve for pressure, we get the second equation, which indicates that to perturb blood pressure we can either alter the flow term or the resistance term, or both. In mammalian organisms, in general, and in man, flow is determined by cardiac output. A crucial aspect of cardiac output is venous return to the heart, and the latter is greatly dependent upon the extracellular fluid volume, especially the vascular volume. This leads to the realization that hypertension has two major etiologic

Correspondence and reprint requests: Jules B. Puschett, MD Scott & White 2401 S. 31st Street, Temple, TX 76508, 254-724-6791, jpuschett@swmail.sw.org

$$\text{Blood Flow (Q)} = \frac{\text{Blood Pressure (P)}}{\text{Resistance (R)}}$$

Or, rearranging:

$$P = Q \times R$$

Cardiac Output (CO) = flow through the systemic circulation; so:

$$BP = CO \times R$$

CO is a function of venous return.

FIG. 1. Flow/Pressure Relationships. The relationship between blood flow (Q), blood pressure (P) and resistance to flow(R) in the vascular circuit.

factors. These are: 1) expansion of the ECF volume, and 2) increased total peripheral resistance. Current estimates are that, in the United States, about 40% of the so-called "essential hypertension", which afflicts in the neighborhood of 60 million Americans, has as its primary etiology, the expansion phenomenon (a consequence of excessive amounts of salt and water), and 50–60% results from increased vascular resistance. Of course, these factors are often *both* involved, especially as the hypertensive state proceeds. As we proceeded to examine these concepts, we realized that nature has provided us with an experiment in volume expansion. It's called pregnancy.

Shown in Figure 2 is what happens to blood volume in pregnant women as gestation proceeds. At term, women have accumulated an additional 40–50% of vascular volume. This brings us to the subject of preeclampsia, which is a syndrome with the characteristics listed in

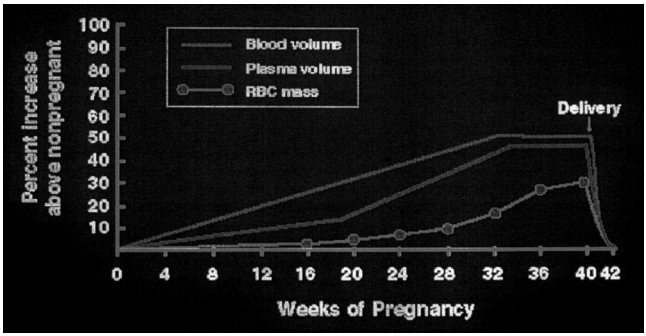


FIG. 2. Alterations in blood and plasma volume and red cell mass during pregnancy. This figure shows what happens to blood volume in pregnant patients as gestation proceeds. At term, animals have accumulated an additional 40–50% of vascular volume. Reproduced (with permission) from Scott DE, Obstet Gynecol Ann 1:219, 1972.

TABLE 1
Characterstics of Preeclampsia

- New onset hypertension (>140/90 mmHg)
- Proteinuria (>300 mg/24 hr)
- Onset after 20 weeks of gestation
- Excessive edema often present
- Intrauterine growth restriction (IUGR) common
- Second leading cause of fetal wastage and maternal morbidity and mortality
- Syndrome remits by 12 weeks postpartum

TABLE 2
Rat Preeclampsia Studies Protocol

- Female Sprague-Dawley rats (250–300 gms)
- Animal groups
  - — Normal controls
  - — Normal pregnant animals
  - — Pregnant animals given saline as drinking water and DOCA injection weekly.
- Sacrificed at 14–16 days of gestation.

Table 1. Preeclampsia is diagnosed when a patient develops *de novo* _hypertension and proteinuria after 20 weeks of gestation. There is often excessive edema formation, and one of the problems that attends this syndrome is the development of intrauterine growth restriction (IUGR). Preeclampsia is the second leading cause of both fetal wastage and maternal morbidity and mortality occurring in 3–10% of all pregnancies (1). The offending organ is the placenta, The only definitive therapy is the delivery of the fetus and placenta. The therapy of this disorder has not changed in over 40 years. Unfortunately, often times,

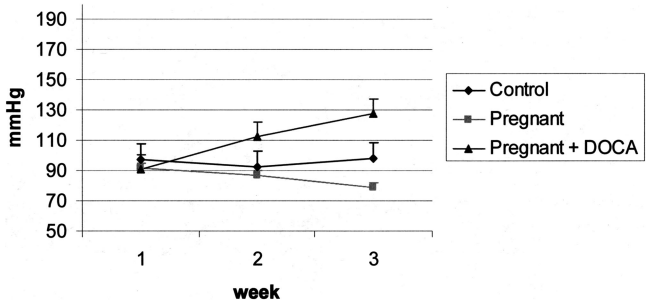


FIG. 3. Blood pressure modification during pregnancy. The effects on blood pressure in normal, control, non-pregnant rats and normal pregnant animals whose drinking water had been replaced with saline and to whom we administered the powerful mineralocorticoid desoxycorticosterone acetate (DOCA). Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

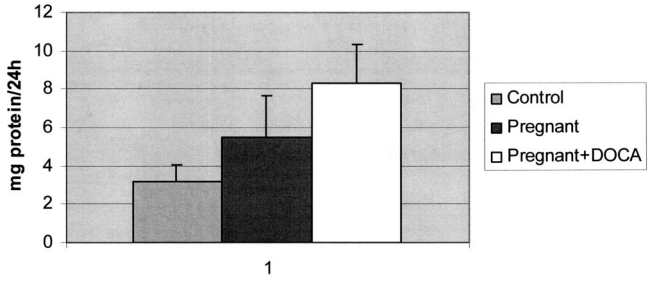


FIG. 4. Proteinuria. Pregnancy was associated with a modest increase in protein excretion over that seen in control, non-pregnant animals, but the protein excretion measured in the PDS rats exceeded that in either of the other two groups. Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

the delivery occurs at a time at which the fetus is not viable or must spend many weeks in the neonatal intensive care unit. Even then, survival is not assured. Remarkably, the hypertension and proteinuria disappear by the 12th week postpartum. We decided to attempt to develop an animal model of preeclampsia based on our postulate that at least some forms of this syndrome represent examples of excessive volume expansion (2).

Our protocol is presented in Table 2. We studied three groups of animals: 1) normal, control, non-pregnant rats, 2) normal pregnant animals (NP) and 3) pregnant animals whose drinking water had been replaced with saline and to whom we had administered the powerful mineralocorticoid, desoxycorticosterone acetate (DOCA), to insure that at least a portion of the excess salt and water was retained. These rats are subsequently labeled PDS.

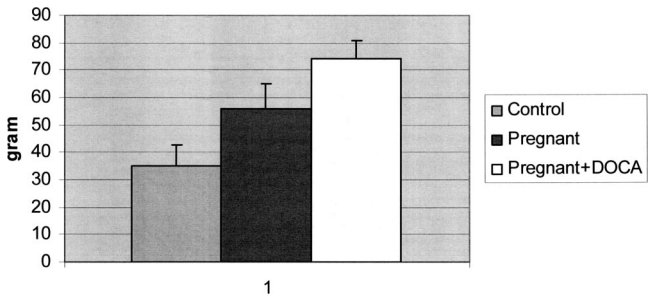


FIG. 5. Weight gain after 2 weeks of pregnancy. At 14–16 days of pregnancy, the PDS rats showed a weight gain that exceeded that of their normal pregnant counterparts, compatible with volume expansion. Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

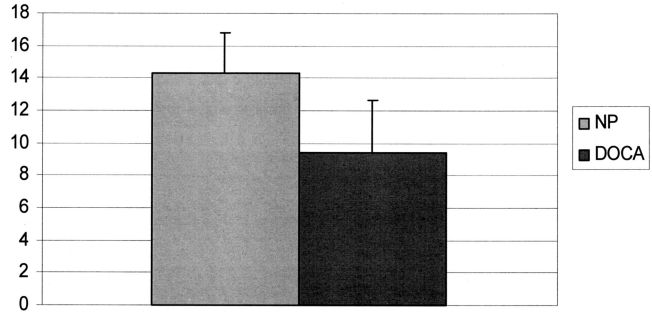


FIG. 6. Pup Number. The number of pups from normal pregnant (NP) and pregnant + DOCA (DOCA) animals were utilized. The difference between the two groups was significant (NP = 14.28 +/−2.56 vs DOCA = 9.43 +/− 3.2, p = 0.002). Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

The effects on blood pressure in these animal groups are shown in Figure 3. As is the case in human pregnancy, blood pressure declined during normal pregnancy, presumably because of the major vasodila-

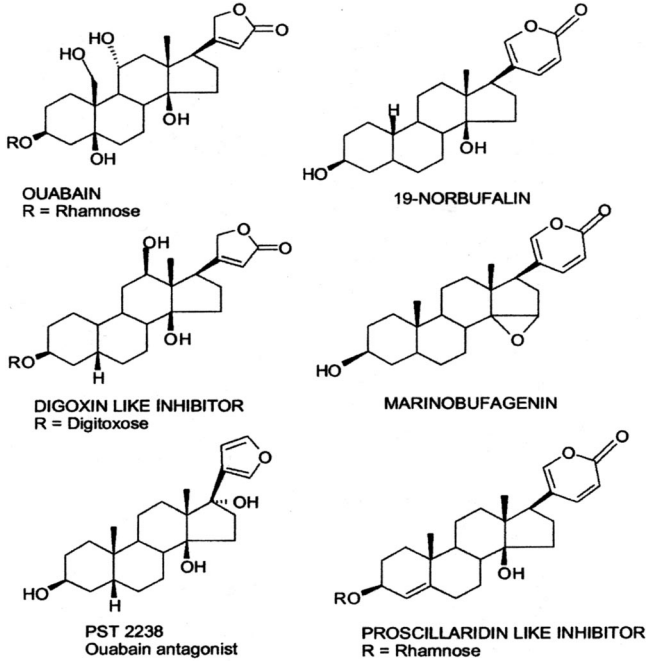
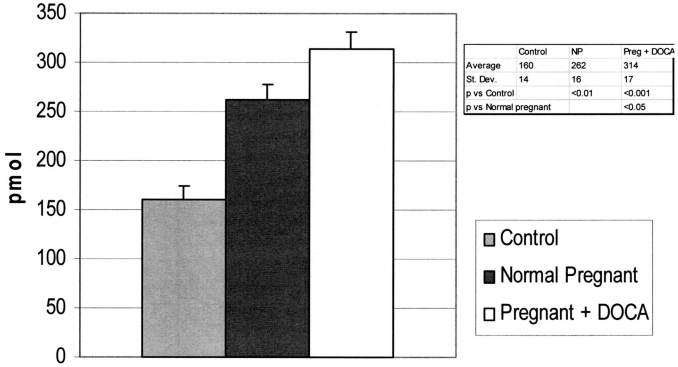


FIG. 7. Endogenous Cardiac Glycosides. Cardenolides (left column) Bufadicnolides (right column).



	Control	NP	Preg + DOCA
Average	160	262	314
St. Dev.	14	16	17
p vs Control		<0.01	<0.001
p vs Normal pregnant			<0.05

FIG. 8. Marinobufagenin Excretion (pmol/24h). An increase in excretion in normal pregnancy and an even greater excretion in our PDS rats was noticed. Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

tation that occurs. The control rats showed no change in the blood pressure, whereas the PDS rats became hypertensive. Pregnancy was associated with a modest increase in protein excretion over that seen in control, non-pregnant animals, but the protein excretion measured in the PDS rats exceeded that in either of the other two groups (Figure 4). At 14–16 days of pregnancy, the PDS rats showed a weight gain that exceeded that of their normal pregnant counterparts, compatible with volume expansion (Figure 5). We used pup number and the appearance of the placenta as measures of IUGR (Figure 6). The pup number was

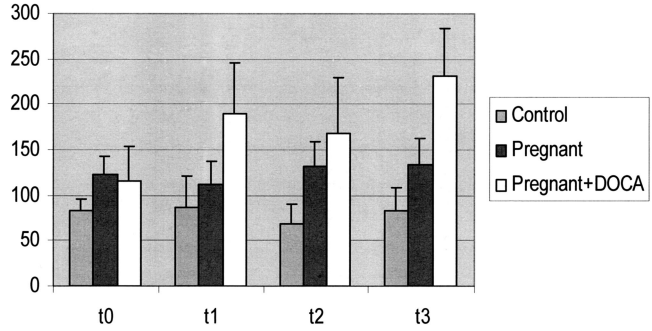


FIG. 9. MGB pmol/24h. Time sequence data for MBG excretion in control, non-pregnant rats, normal pregnant animals and PDS rats. Time 0 represents baseline measurements. Time T1 depicts measurements performed after 5–6 days of pregnancy. T2 represents 13–14 days of pregnancy and the T3 observations were determined at 19–21 days of pregnancy, just before delivery. Reproduced (with permission) from Ianosi-Irimie M, Vu HV, Whitbred JM, et al. Clin Exp Hypertens 2005; 8:605–17.

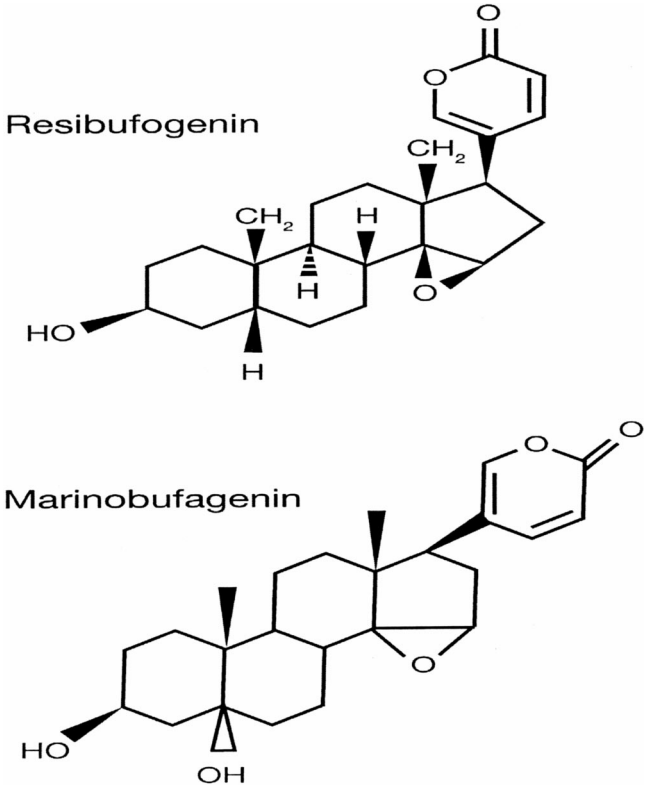
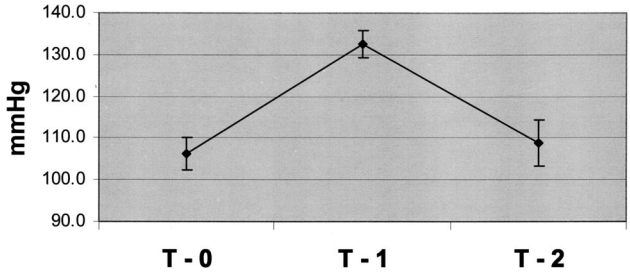


FIG. 10. Resibufogenin (RBG) and marinobufagenin (MBG) chemical structures. RBG differs from MBG only in the absence of an hydroxyl group at the beta-5 position. Reproduced (with permission) from Vu H, Ianosi-Irimie M, Danchuk S, et al. Exp Biol Med 2006; 231:215–20.

significantly less in our PDS rats than in normal pregnancy. We frequently saw areas in the placenta of the PDS rats that appeared to represent either aborted or absorbed fetuses. In addition, there were examples of fetal malformation. These observations were not present in our normal pregnant animals.

Let me digress now to discuss a group of compounds called cardiac glycosides in an attempt to communicate why we believe that one or more of these compounds may play a role in preeclampsia. The cardiac glycosides consist of two groups: the cardenolides and the bufodienolides (Figure 7) (3). These compounds circulate in the blood. The archetypical cardenolides are oaubain and digoxin. The bufodienolides were first detected in the skin and venom of the toad, *Bufo marinus.* The most extensively studied of the bufodienolides is marinobufagenin



Mean BP	106.3	132.5	108.8
St. Error	3.8	3.2	5.5
		p < 0.05	p < 0.05

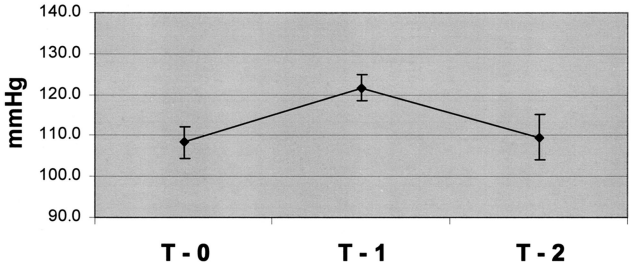
FIG. 11. PDS + RBG, RBG was administered to our PDS animals and a return of the blood pressure to normal was seen. Reproduced (with permission) from Vu H, Ianosi-Irimie M, Danchuk S, et al. Exp Biol Med 2006; 231:215–20.

(MGB). All of these compounds are inhibitors of Na^+/K^+ ATPase, although the bufodienolides act primarily on the alpha-1 isoform of the enzyme, while the cardenolides have a predilection for the alpha-2 and-3 isoforms. They cause hypertension, natriuresis and are cardiac inotropes. Their secretion and elaboration are stimulated by volume expansion.

In collaboration with a group of investigators at the National Institute on Aging, we measured the urinary excretion of MBG in our animals (4). We noticed an increase in excretion in normal pregnancy and an even greater excretion in our PDS rats (Figure 8). Blood levels of MBG have been reported to be elevated in preeclamptic patients.

Presented in Figure 9 are time sequence data for MBG excretion in control, non-pregnant rats, normal, pregnant animals and PDS rats. Time 0 represents baseline measurements. Time T1 depicts measurements performed after 5–6 days of pregnancy. T2 represents 13–14 days of pregnancy and the T3 observations were determined at 19–21 days of pregnancy, just before delivery. The average gestation period in the rat is 20–21 days. MBG excretion in the PDS animals increased at time T1, over those seen in the other two groups of animals, and remained increased for the balance of the pregnancy. However, interestingly, at time T1, when urinary MBG excretion is already increasing, the rats are *not yet* hypertensive or proteinuric. These observations suggested to us that MBG is at least a potential marker of the later development of a syndrome that resembles human preeclampsia, or is pathogenetically involved in the process, or both.

Additionally, we have determined that there is an antagonist of



Mean BP	108.3	121.7	109.6
St. Error	4.2	4.2	3.2
		p < 0.01	p < 0.01

FIG. 12. MBG + RBG. MBG was given intraperitoneally to normal pregnant rats which rendered them hypertensive. RBG was then given to these animals and the blood pressure was observed to have returned to normal levels. Reproduced (with permission) from Vu H, Ianosi-Irimie M, Danchuk S, et al. Exp Biol Med 2006; 231:215–20.

MBG (5). Shown in Figure 10 is the structure of a compound called resibufogenin (RBG). RBG differs from MBG only in the absence of an hydroxyl group at the beta-5 position. We administered RBG to our PDS animals and saw a return of the blood pressure to normal (Figure 11) and a reduction in proteinuria (data not shown). We gave MBG intraperitoneally to normal pregnant rats and rendered them hypertensive. We then gave RBG to these animals and observed the return of the blood pressure to normal levels (Figure 12) (5).

In summary, in our rat model of human preeclampsia, MBG plays a pathogenetic role and/or is utilizable as a predictor of the later development of the syndrome. Furthermore, an antagonist of MBG has been discovered (resibufogenin, RBG) which may also have clinical application. These observations have led us to examine these theses in patients with preeclampsia. Those studies are currently underway.

ACKNOWLEDGMENT

This work was supported, in part, by a grant-in-aid from Dialysis Clinic, Inc. and by the Louisiana Board of Regents Millennium Health Excellence Fund (2001-2006)-07. During the tenure of this work, Dr. Puschett was a member of the Tulane Hypertension and Renal Center of Excellence. The author thanks Mrs. Lonnie Doyle for the production of this manuscript.

REFERENCES

1. Pridjian G, Puschett JB. Preeclampsia, Part I: Clinical and pathophysiological considerations. Obstetrical and Gynecological Survey 57:598–618, 2002.

2. Ianosi-Irimie M, Nadig JD, Williams MW, et al. A rat model of preeclampsia. Clin Exp Hypertens 8:605–617, 2005.
3. Schoner W. Endogenous cardiac glycosides, a new class of steroid hormones. Eur J Biochem 2002; 269:2440–8.
4. Vu H, Ianosi-Irimie M, Pridjian C, et al. Involvement of marinobufagenin in a rat model of human preeclampsia. Am J Nephrol 25: 520–528, 2005.
5. Vu H, Ianosi-Irimie M, Danchuk S, et al. Resibufagenin corrects hypertension in a rat model of human preeclampsia. Exp Biol Med 231: 215–220, 2006.

DISCUSSION

Thorner, Charlottesville: That was a beautiful presentation. Do you have any sense where MBG is made? There was a story several years ago suggesting that the other part of the family was made in the posterior pituitary, and I wonder if that is a potential source?

Puschett, Temple: Thank you. I don't have any information on the posterior pituitary. We do know it is made in the adrenal and in the placenta for sure, and we are looking at other organs now to see if we can verify where else it might be made.

Alpert, Tucson: I followed this literature some years ago, and I remember there was a fair amount of interest in decreased levels of vasodilatory prostaglandins in the placenta as a possible explanation. Has that been discredited?

Puschett, Temple: I don't think it is discredited, but it hasn't received a lot of attention recently. You know, the thing about preeclampsia is that it has so many potential etiologic factors that it is hard to separate them out sometimes. I think most of us are now convinced that preeclampsia is not a single disease; like essential hypertension, it has a lot of etiologic factors. We have done a chart study recently that suggests that our preeclamptic rat model may be looking a lot like a significant number of patients with preeclampsia. We unfortunately lost about 350 samples from patients with preeclampsia in the storm. So, we have had to start all over again, but hopefully some day, we will be able to answer that question.